survival signaling by BPs, Cx43 is dispensable for cellular BP uptake, BP binding and proliferative effects.

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Osteocyte-selective deletion of CX43 results in enhanced response to mechanical loading in mice

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The gap junction protein connexin43 (Cx43) mediates the communication of osteocytes among themselves and with the extracellular milieu. Although Cx43 appears to be important for the response of osteocytic cells to mechanical stimulation *in vitro*, the contribution of Cx43 in osteocytes to mechanotransduction *in vivo* is unknown. To this end, we examined the anabolic response to loading of mice lacking Cx43 in osteocytes (Cx43ΔOT) generated by crossing Cx43fl/fl mice with 8kbDMP1-Cre mice. Effective deletion of Cx43 was confirmed by an 88% decreased in Cx43 mRNA in osteocyte-enriched cortical bone from Cx43ΔOT mice, compared to control mice. Right ulnae were subjected to axial loading at equivalent low, medium, and high strain magnitudes during 1 min/d for 3 days; left ulnae were used as non-loaded controls. Bone formation rate (BFR/BS), quantified 14 d later showed that loading induced strain-dependent increases in periosteal BFR/BS of 1.4, 1.8, and 2.2 fold in control mice. To our surprise, loading induced a greater response in Cx43ΔOT mice (2.5, 4.3 and 4.9 fold) due to a combination of higher mineralizing surface and higher rate of mineral apposition. These results suggest that the lack of Cx43 in osteocytes unleashes bone formation by periosteal osteoblasts and are consistent with the higher basal periosteal bone formation in femora from Cx43ΔOT mice. The apparent discrepancy with the decreased endosteal BFR/BS induced by tibia loading in mice lacking Cx43 in both osteocytes and osteoblasts (2.3kbcol1a1) can be explained by the fact that, in the latter model, osteoblasts are unable to function optimally. We conclude that the intrinsic function of Cx43 in osteocytes is to restrain the response of osteoblasts to mechanical loading.

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Protective role of 17B-estradiol in apoptosis of skeletal muscle

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We previously described a non classical localization for both estrogen receptors (ERs) in the C2C12 cell line and in mouse skeletal muscle tissue. ERβ was exclusively detected in mitochondria and ERα in the perinuclear and mitochondrial compartments. Since mitochondria are key organelles in apoptosis, this subcellular location of ERs could be associated with the regulation of apoptosis of muscle cells by 17β-estradiol (E2). Thus, we demonstrated that E2, at physiological concentrations, abrogates H_2O_2 induced-apoptosis through both ERα and ERβ. Moreover, the mechanism activated by E2 in this protective action involved PI3K/Akt/Bad and MAPKs pathways and HSP27. We found that the steroid abrogates DNA damage, PARP and caspase-3 cleavage, cytochrome c and Smac/DIABLO release from mitochondria, induced by H_2O_2. Now, we perform flow cytometry studieswith the cationic dye JC-1, showing that H_2O_2 induces a decrease in mitochondrial membrane potential, which is prevented by E2. In view that the loss of mitochondrial membrane potential could be due to the continuous mitochondrial permeability transition pore (MPTP) activation, we evaluated its functionality with calcein-AM by microscopy. We evidenced loss of green mitochondrial calcein fluorescence in cells treated with H_2O_2. However, this loss of fluorescence was prevented when the cells were preincubated with E2 and then treated with H_2O_2. Our studies have shown an important role of E2 in the regulation of apoptosis in muscle cells with a clear action at mitochondrial level. These findings could be important to overcome myopathies due to dysregulated apoptosis by disorders in estrogen metabolism.

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Involvement of the NFKB pathway in 1A,25(OH)2-vitamin D3-induced growth inhibition of Kaposi sarcoma

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Epidemiologic and clinical studies have shown that the hormonal form of vitamin D3, 1α,25(OH)2-vitamin D3 (1α,25(OH)2D3), in addition to playing a central role in the control of calcium homeostasis, participates in several molecular mechanisms involved in tumoral transformation of cells. We have previously demonstrated that the hormone has antiproliferative effects on the growth of endothelial cells transformed by the viral G protein-coupled receptor associated to Kaposi sarcoma (SVEC-vGPCR). In this work, we have investigated the mechanism by which 1α,25(OH)2D3 exerts its growth inhibitory effects. Time and dose response studies, by QRT-PCR and Western blot showed that the hormone significantly decreased NFkB and increased IkBα RNA and protein levels in SVEC-vGPCR, whereas in non-transformed cells only IkBα increased significantly. The most potent effect was observed at 16 h and 10 nM 1α,25(OH)2D3. Moreover, confocal microscopy studies showed that NFkB translocation to the nucleus was inhibited and occurred by a mechanism independent of NFkB association with the vitamin D3 receptor (VDR). Hormone-induced increase in IκBα required *de novo* protein synthesis, and was independent of MAPK and PI3K/Akt pathways. Altogether, these results suggest that the antiproliferative effects of 1α,25(OH)2D3 in Kaposi sarcoma's cellular model take place by down-regulation of the NFkB pathway.

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Role of androgen receptor in the protective action of testosterone during apoptosis in skeletal muscle

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Apoptosis occurs in response to environmental or developmental cues, cellular stresses and specific cell death signals. We have previously demonstrated that testosterone protects against H_2O_2-induced apoptosis in C2C12 muscle cells. Typical changes of apoptosis such as nuclear fragmentation, cytoskeleton disorganization, mitochondrial reorganization/dysfunction and cytochrome c release induced by H_2O_2, are abolished when cells are previously exposed to the hormone. Also, the molecular events activated, especially those that involve the mitochondria as a target, have begun to be elucidated. In the present work, we demonstrated not only a non-classical localization of the androgen receptor (AR) but also its contribution to the anti-apoptotic effect of testosterone. Treatments with the AR antagonist, flutamide, prior to testosterone and H_2O_2 exposure, reduce the protective effect of the hormone. We confirmed it by the evaluation of the expression level, phosphorylation states and subcellular localization of ERK1/2 MAPKs, Akt, 14-3-3, members of the Bcl-2 family and PARP. The mitochondrial membrane potential was measured by flow cytometry, using the fluorescent dye JC-1. These studies showed the participation of AR in the protective role of testosterone. Moreover, we could demonstrate a non-classical localization of AR in mitochondria and microdomains (caveolae and rafts) by competitive binding assays, immunocytochemistry and immunoblottings of subcellular fractions and gradient centrifugation. The results point to an active role of AR during the anti-apoptotic effect of testosterone at the nuclear (genomic response), mitochondrial (intrinsic pathway) and microsomal (response mediated by membrane proteins) levels.

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Vertebral fracture in patients with Gaucher disease

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Gaucher disease (GD) affects the bone marrow and is associated with osteopenia, osteoporosis and osteonecrosis, but little information on vertebral fractures (VF). **Objective:** To detect by X-ray the presence of asymptomatic fractures in patients with GD. **Materials and methods:** 15 patients (2 men, 13 women; mean age, 44.8 years (20–72) with GD were evaluated. Diagnosis mean time was 15.46 years (4–30). All patients underwent a lateral dorsal spine (DS) X-ray focused on D8 and a lumbar spine (LS) focused on L3 and were analysed according to Genant's semi-quantitative classification for fracture: grade I to be up to a 25% reduction in the height of the anterior, middle or posterior vertebral body, a grade II reduction in height of 25–40%, and a grade III reduction in height of more than 40%. Patients found to have a VF underwent DXA to assess bone mineral density (BMD). They were asked whether they experienced pain in the DS and LS, and if they knew they had sustained a VF. **Results:** 26.66% of the patients (4/15) presented VF: patient 1: premenopausal, fracture at L4 grade I; patient 2: premenopausal, fractures at L2 and T12, both grade I; patient 3: postmenopausal, fracture at L5 grade I and patient 4: postmenopausal, fractures at L3 and L1, both grade II. BMD evaluation could not be performed for one of the patients because she had undergone spine and bilateral hip surgery. BMD was with within the normal range for sex and age for the other three patients. When questioned, patients who had fractures did not report specific pain in the DS or LS, and did not know that they had a vertebral fracture. **Conclusion:** VF is often asymptomatic and in addition, patients with GD may not report pain. Therefore, to ensure that all VF are detected, DS and LS X-rays should be performed.

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